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Developing drug-induced resistant tumor models for efficacy evaluation of next-generation anticancer therapies

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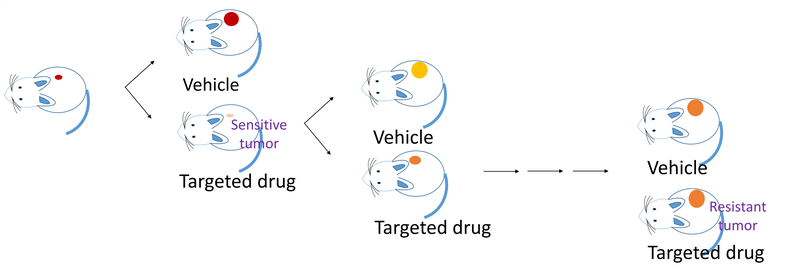
Abstract

The therapeutic landscape of cancer has been transformed over the last few decades. Our understanding of cancer and its therapeutic approach has improved greatly due to advanced cancer biology, functional imaging and next–generation sequencing. One of the key challenges in cancer therapy is how to effectively kill cancer cells while leaving normal cells intact. As specific oncogenic drivers have been successfully identified, targeted treatments have achieved great success in clinical practice. However, acquired drug resistance remains the major barrier to sustained success of targeted cancer therapy. Those patients who initially respond well to targeted therapies eventually become resistant to the treatments. Uncovering the underlying mechanism would help to develop novel therapies that overcome the drug resistance.

To mimic the clinical resistance after long-term drug treatment, we established a panel of drug-induced resistant tumor models by continuous dosing of targeted drugs to tumor-bearing mice or cancer cell lines, covering a series of first-line targeted drugs including Sotorasib, Palbociclib, Ibrutinib, Capmatinib, Fulvestrant, Tamoxifen and T-DM1. Resistant models were derived from sensitive ones harboring specific oncogenic drivers. After treated with targeted drugs and passaged several times, resistant models exhibited stable resistance phenotype. Given that target gene mutation, alternative pathway activation or tumor microenvironment evolution could promote therapy resistance and cancer progression, we performed transcript and protein analysis to explore the molecular mechanism, and evaluated the potential therapeutic strategies overcoming drug resistance in the established models. Taken together, drug-induced resistant tumor models provide a promising opportunity to the better understanding of drug resistance mechanisms and to the accelerated development of next-generation anticancer agents.

Method

In vivo



In vitro

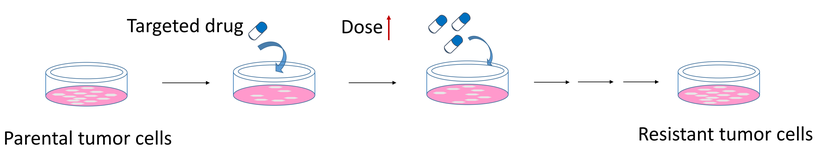


Fig 1. Generation of targeted drug-induced resistant tumor models *in vivo* and cell lines *in vitro*
Parental tumors harboring oncogenic drivers exhibited significant sensitivity to antitumor targeted drugs. After chronic dosing to tumor-bearing mice or tumor cell lines, regrowth tumors were continuously treated and implanted (subcultured) to the next passage until a stable drug resistance phenotype occurred.

Results

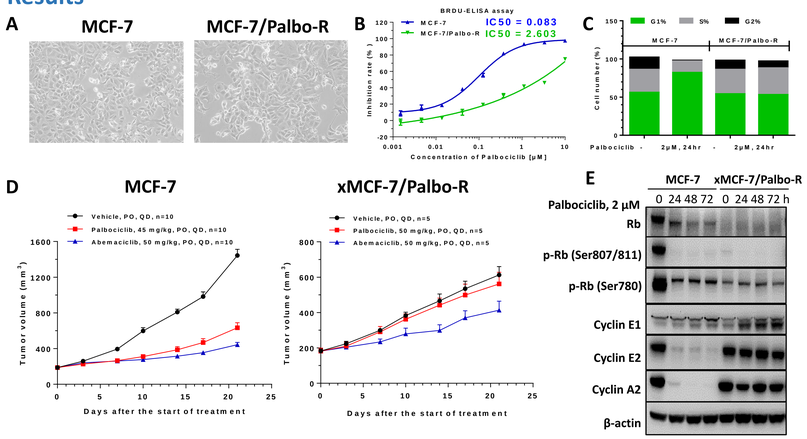


Fig 2. *In vitro* induced Palbociclib resistant MCF-7 cell line
Palbociclib, a CDK4/6 inhibitor, has been approved for advanced or metastatic HR+, HER2- breast cancer. **A.** MCF-7/Palbo-R cell line was established through chronic exposure to increased concentrations of Palbociclib (up to 4 μM) for ~7 months. **B.** MCF-7/Palbo-R cell line was resistant to Palbociclib *in vitro*. **C.** Palbociclib failed to induce cell cycle arrest in MCF-7/Palbo-R cell line. **D.** xMCF-7/Palbo-R cell line was derived from MCF-7/Palbo-R tumors. xMCF-7/Palbo-R model was resistant to Palbociclib *in vivo*. **E.** Rb expression level was markedly decreased in xMCF-7/Palbo-R cell line, which might contribute to resistance.

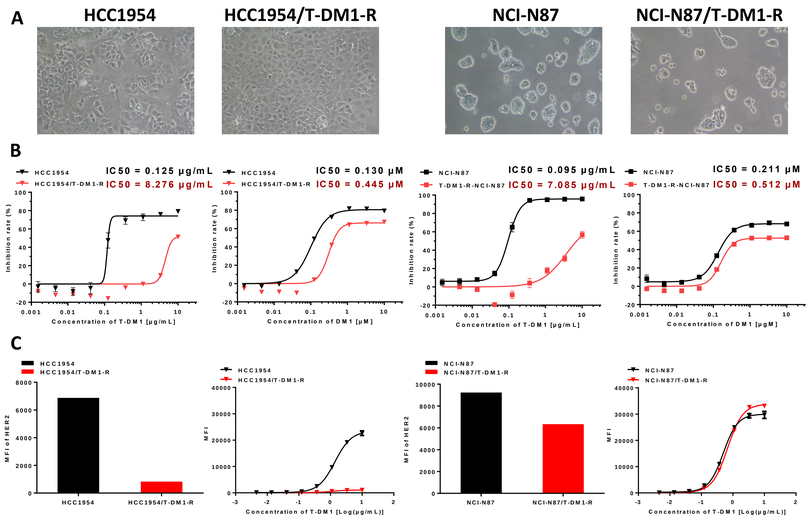


Fig 3. *In vitro* induced T-DM1 resistant HCC1954 and NCI-N87 cell lines
T-DM1 was a HER2-targeted ADC, approved for metastatic HER2+ breast cancer.
A. HCC1954/T-DM1-R and NCI-N87/T-DM1-R cell lines were established by chronic treatment with increased concentrations of T-DM1 (up to 6 μg/mL) for ~8 months. **B**. HCC1954/T-DM1-R and NCI-N87/T-DM1-R cell lines were resistant to T-DM1 *in vitro*, but not DM1. **C**. In HCC1954/T-DM1-R cell line, HER2 expression level and the binding activity between HER2 and T-DM1 significantly reduced, which could be a possible mechanism of resistance. In NCI-N87/T-DM1-R cell line, there was no significant difference in HER2 expression as well as its binding with T-DM1, suggesting a HER2-independent resistant mechanism.

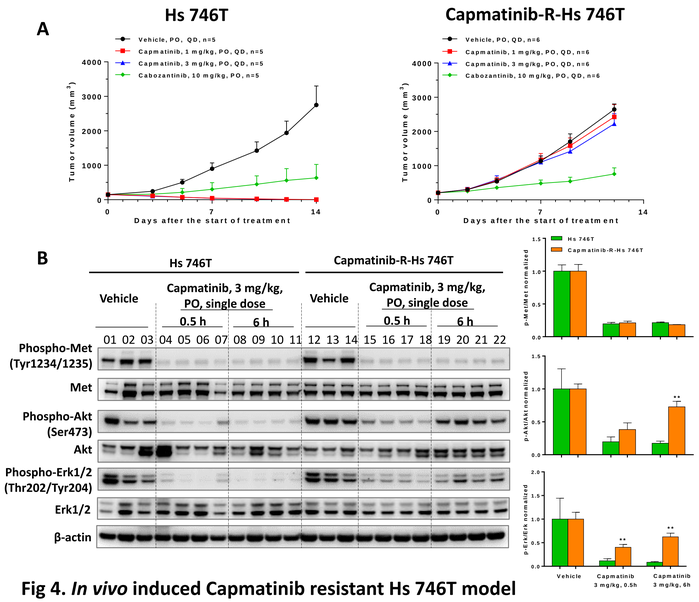


Fig 4. *In vivo* induced Capmatinib resistant Hs 746T model
Capmatinib is a highly selective MET inhibitor to treat non-small cell lung cancer carrying MET exon 14 skipping mutation. **A.** Capmatinib-R-Hs 746T model was resistant to Type Ib MET TKI Capmatinib, while was sensitive to Type II MET TKI Cabozantinib. **B.** In Capmatinib-R-Hs 746T model, Capmatinib produced less inhibition effects on p-Akt and p-Erk1/2 compared with Hs 746T model.

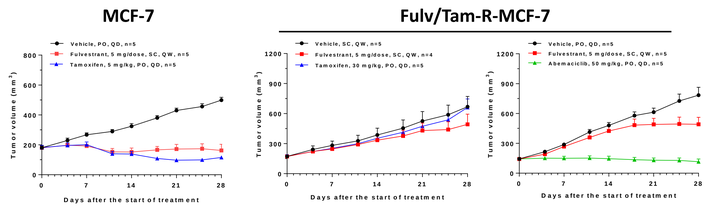


Fig 5. *In vivo* induced Fulvestrant/Tamoxifen resistant MCF-7 model
Both Fulvestrant and Tamoxifen are targeted to estrogen receptor, producing antagonistic effects by different mechanisms of action. Fulv/Tam-R-MCF-7 model is resistant to both Tamoxifen and Fulvestrant, and still sensitive to Abemaciclib.

Summary

We have successfully established a series of *in vivo/vitro* induced drug resistant models by continuous dosing of targeted drugs, evaluating the antitumor effect of potential drugs in the same or different pathways. Furthermore, we performed transcript-level and protein-level analysis in the parental and resistant models to explore the resistant mechanisms.

Reference

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